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APPLICATION FOR UNITED STATES PATENT

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Title: METHOD FOR TREATING CYTOKINE MEDIATED HEPATIC INJURY

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Att Dock #: ZYM-09US

Specification

METHOD FOR TREATING CYTOKINE MEDIATED HEPATIC INJURY

This application claims the benefit of United States Application Serial No. 60/238,991, filed October 10, 2000.

Field of the Invention

The invention relates to the use of compounds to attenuate or prevent cytokine mediated hepatic injury.

Background

Hepatic injury can be caused by a number of different agents including viruses such as Hepatitis A, B, C, D and E, both gram positive and gram negative bacteria, chemical agents such as ethanol, carbon tetrachloride and lead, and by physical trauma resulting in ischemia (ischemic hepatitis) injuries as can occur in right-sided congestive heart failure. It is now believed that all of these types of hepatic injury are caused at least in part by the liver's inflammatory or cytokine response to these agents. The inflammatory response of the liver results in the overexpression of a cascade of inflammatory/acute phase cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-6, IL-8 and transforming growth factor beta (TGFβ). It is now believed that it is the cascade of these cytokines which is the ultimate cause of much of the hepatic injury resulting from these agents. Thus, there is

a need for a therapeutic agent which can be useful in alleviating or modulating the inflammatory response associated with liver disease or injury.

Summary of the Invention

The present invention fills this need by providing a method of treating or preventing a cytokine mediated hepatic injury in a mammal comprised of administering a pharmaceutically effective amount of a peptide having the sequence Tyr-D-Leu-Phe-Ala-Asp-Val-Ala-Ser-Thr-Ile-Gly-Asp-Phe-Phe-His-Ser-Ile-NH_2 SEQ ID NO: 1, hereinafter referred to as compound D, to said mammal. The hepatic injury can be an acute inflammatory reaction, as a result of a viral or bacterial infection or a chemical agent such as ethanol, lead, carbon tetrachloride or acetaminophen, or from trauma resulting in ischemia or reperfusion injury in the liver.

The present invention is also directed to a method of treating a viral or bacterial infection-related hepatic damage in a mammal comprised of administering a pharmaceutically effective amount of compound D SEQ ID NO: 1 to said mammal.

The present invention is also directed to a method of treating alcohol induced liver injury in a mammal comprised of administering a pharmaceutically effective amount of compound D SEQ ID NO: 1 to said mammal.

Preferably, compound D SEQ ID NO:1 is administered in a pharmaceutical composition at a dosage of from about 0.5 mg/kg to about 20 mg/kg per body weight of the mammal.

Preferably, the mammal is a human.

Detailed Description

A compound used to treat cytokine-mediated hepatic injury is a peptide having the sequence Tyr-D-Leu-Phe-Ala-Asp-Val-Ala-Ser-Thr-Ile-Gly-Asp-Phe-Phe-His-Ser-Ile-NH_2 SEQ ID NO:1, hereinafter referred to compound-D. The peptide may be produced by a number of methods, such as using an automated peptide synthesizer, through recombinant molecular techniques, or isolated from a naturally occurring source, as is known to one skilled in the art. Compound-D SEQ ID NO:1 has a molecular weight of 1,902 daltons. Compound-D SEQ ID NO:1 is insoluble in water or saline, but may be solubilized by adding 100 µM of a solution comprised of ethanol, propylene glycol, and 1 N NaOH in a 1:1:1 ratio, with sterile physiological saline then used to obtain the appropriate concentration. The initial alkaline pH is adjusted to 7.4 with 1 N HCl.

Compound-D SEQ ID NO:1 that has been solubilized may be administered by parenteral means, for example, by intravenous injection. For administration into a mammal, a dose of about 1-20 milligrams per kilogram (mg/kg) is useful. For administration into a tissue or organ preservation solution, a concentration of about 100 µM is useful.

Compound-D SEQ ID NO:1 may be administered directly into a mammal, either alone or in combination with other substances.

The above agent is administered to a mammal to modulate cytokine activation by blocking one or more steps in the cytokine cascade. The agent may be formulated for administration in an aqueous based liquid such as phosphate buffered saline to form an emulsion, or may be formulated in an organic liquid such as dimethylsulfoxide to form a solution. The solution or

emulsion may be administered by any route, but it is preferably administered parenterally such as by intravenous, intramuscular, intradermal or intraperitoneal injections. A preferred dose is in the range of about 0.5-20 mg of compound-D SEQ ID NO:1 per kg of body weight of the mammal. The time of administration of the agent is preferably prior to initiation of cytokine activation. However, the agent may be administered concurrently with another agent that induces cytokine activation or even subsequent to an agent that induces cytokine activation and still produce a protective effect.

Administration of compound-D SEQ ID NO:1 should be continued on a daily basis until hepatic function returns to normal and is maintained at normal levels, preferably for at least one to two days. Hepatic injury can be determined by elevated levels of hepatic enzymes, as well as by depressed albumin levels (less than about 35 g/liter). Hepatic function is routinely monitored by quantitating serum levels of hepatic enzymes such as alanine aminotransferase (ALT) (normal < 35 U/L), aspartate aminotransferase (AST) (normal < 30 U/L), alkaline phosphatase (ALP) (normal $\leq$ 100 U/L) and gamma glutamyltransferase (GGT) (normal $\leq$ 45 U/L for males, $\leq$ 30 U/L for females), as well as bilirubin, both conjugated (normal $\leq$ 0.2 mg/deciliter) and total (normal $\leq$ 1.0 mg/deciliter) bilirubin. Compound-D SEQ ID NO:1 modulation of hepatocyte cytokine activation may be used therapeutically in a variety of hepatic injury processes. As used herein, the term hepatic injury broadly encompasses all types of injury such as hepatic trauma, physical and/or chemical insult, stress, inflammation, toxicity, disease and so on. For example, the inventive agents can be used in treating hepatic injury due to alcoholic liver disease, acetaminophen toxicity, cadmium toxicity, lead poisoning, bacteremia

due to, for example, *Staphylococcus* species, *Streptococcus* species, *Neisseria* species, *Salmonella* species, *Shigella* species, *Escherichia coli*, *Clostridium perfringens*, *Klebsiella* species, *Proteus* species, *Enterobacter* species, *Bacteroides* species, *Brucella* species, *Francisella tularensis*, *Listeria monocytogenes*, *Acinetobacter* species, *Streptobacillus moniliformis*, *Vibrio* species, *Helicobacter pylori*, *Pseudomonas* species, *Haemophilus* species, *Bordetella pertussis*, viral infections due to, for example, influenza viruses, adenoviruses, paramyxoviruses, rubella viruses, polioviruses, hepatitis viruses, herpesviruses, rabies viruses, human immunodeficiency viruses and papilloma viruses, as well as trauma, ischemia reperfusion injury and metabolic liver disease.

While the specific mechanism of action of compound-D SEQ ID NO:1 on the modulation of cytokine mediated hepatic injury such as acute inflammatory reactions, trauma and toxin induced biological responses is unknown, these agents exhibit a specific and reproducible effect on decreasing hepatotoxicity.

A treatment for attenuating and/or preventing cytokine mediated acute inflammatory, trauma induced and toxin induced hepatic injury is thus disclosed. Compound-D SEQ ID NO:1, administered at a concentration of about 0.5 mg/kg to about 20 mg/kg, inhibits hepatic injury and result in decreased lethality of an injured animal.

It should be understood that the embodiments of the present invention shown and described in the specification are only preferred embodiments of the inventors who are skilled in the art and thus are not limiting in any way. Therefore various changes, modifications or alterations to these

embodiments may be made or resorted to without departing from the spirit of the invention and the scope of the following claims.

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<120> METHOD FOR TREATING CYTOKINE MEDIATED HEPATIC INJURY

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<223> Xaa = D-Leu; artificial sequence is completely synthesized

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